the theories are limited. We have developed novel microfluidic devices to understand and question whether the binding of AFPs to ice surfaces is irreversible or reversible. Single ice crystals (~20-100 μm) grown in AFP solution can be kept in this controlled environment in which one is able to adjust the concentration of the protein that is in the solution and the temperature of the cell itself. We have tested two different hyperactive AFPs from spruce budworm and *Tenebrio molitor*; both are beta-helical and have a good shape complementary to ice surfaces. We demonstrate AFPs which are attached to ice crystals keep protecting the crystal once the flow turned on and solution is exchanged with AFP free buffer solution. We followed crystals in this AFP free buffer solution for hours within a constant temperature gradient. Our observations show that there is neither growth nor melting of the crystal observed in this continuous flow of buffer solution, and there cannot be an exchange of AFPs between solution and ice surfaces as it was claimed by some in the literature. Based on fluorescence microcopy and microfluidic devices, we conclude that antifreeze proteins from spruce budworm (sbwAFP) and *Tenebrio molitor* (TmAFP) are adsorbed to ice surfaces irreversibly, and thus our observations are in line with adsorption-inhibition theory.

2813-Plat
Dynamic and Strucutral Effects of Ligand and Coregulator Binding on Estrogen Receptor Ligand Binding Domain Measured by Electron Paramagnetic Resonance
Stefano V. Gulla.
Northeastern Univeristy, Boston, MA, USA.
The estrogen receptor (ER) is an important therapeutic target for the treatment and prevention of estrogen responsive forms of breast cancer. Despite the availability of several crystal structures for ER bound to either agonist or antagonist ligands, its molecular mechanism of action still remains unclear. The major structural difference between agonist and antagonist forms can be observed in the position of helix-12 (H12) C-terminus region. Here, we present the results of site directed spin labeling on the H12 region (543) and on the H11-H12 hinge region (530) to monitor the effect of ligands with different biological activity on the solution dynamic and structure of H12. We found that the hinge region is directly affected by allosteric binding of coregulators peptides in a ligand dependent fashion. We characterized the structural changes resulting from ligand/coregulator binding using DEER spectroscopy. Additionally the effects of ligand binding on H12 were directly observable with our 543 labeled ER. When taken together, these results substantially complete our current understanding of the interplay between ligand/coregulator binding and dynamic/structural changes that regulate ER's biological activity.

2814-Plat
Structural Changes And Binding Kinetics Of Fluoro-tryptophan Substituted HyHEL-10 scFv Monitored Using 19F-NMR, High Resolution Crystal Structures And SPR-Biacore Analysis
Mauro Acchione, Morgan Desantis, Yi Chien, Mi Li, Claudia Lipschultz, Aranganathan Shanmuganathan, Joe Barchi, Alex Wlodawer, Sandi Smith-Gill.
National Cancer Institute, Frederick, MD, USA.
High affinity and specificity are hallmarks of the observed association of an antibody with its antigen. These characteristics are governed by a combination of non-bonded interactions and shape complementarity at the binding interface. The flexibility of the CDR binding loops plays a key role in the ability of the antigen to adapt to the surface of the antigen. We explored these relationships using 19-F fluorine NMR by measuring chemical shifts and T2 relaxation parameters for the binding of HyHEL-10 scFv antibody to hen eggwhite lysozyme and epitope-specific mutants. By incorporating 5-fluorotryptophan into the scFv we had a total of 6 NMR sensitive probes in its structure. Individual replacement of those residues with phenylalanine allowed for assignment of each peak in the NMR spectrum so that changes in signal could be analyzed in a site-specific way. A residue-specific analysis is shown including the structural changes occurring during binding. Analysis of binding kinetics using SPR (Biacore) coupled with high resolution crystal structures for the complex showed an unexpected and interesting impact of the 5-fluorotryptophan incorporation on binding affinity. Together these results provide new insights into the underlying structural and dynamic characteristics for tight association and high specificity in biomolecular protein interactions.

2815-Plat
Kinetics and Thermodynamics of Antibody Binding to B-Type Natriuretic Peptide
Sergey Y. Tetin, Qiaoqiao Ruan, Sylvia C. Saldana.
Abbott Laboratories, Abbott Park, IL, USA.
B-type natriuretic peptide (BNP) is a naturally secreted regulatory hormone that influences blood pressure and vascular water retention. The plasma BNP concentration is a clinically recognized biomarker for various cardiovascular diseases. Quantitative detection of BNP can be achieved in immunoassays using high-affinity monoclonal antibodies. Temperature dependence of the equilibrium binding constants and the kinetic rates were studied for anti-BNP mAbs 106.3 and 3-631 by means of fluorescence spectroscopy. Thermodynamic parameters including changes in the free energy, enthalpy and entropy measured at equilibrium are in a good agreement with the parameters calculated from kinetics data. The differences in thermodynamic parameters measured for the two antibodies under study support structural data obtained by NMR and X-ray crystallography.

2816-Plat
Allosteric Regulation Across a β-Sandwich Protein: How a Bacterial Adhesive Protein is Activated by Mechanical Force
Wendy E. Thomas[1], Isolde Le Trong[1], Pavel Aprikian[1], Manu Forero[2], Brian Kidd[1], Gianluca Interlandi[1], Veronika Tchesnekova[1], Olga Yakovenko[1], Viola Vogel[3], Ron Stenkamp[1], Evgeni Sokurenko[1].
[1]University of Washington, Seattle, WA, USA, [2]Universidad de los Andes, Bogota, Colombia, [3]ETH Zurich, Zurich, Switzerland.
We have previously proposed that allosteric regulation causes some receptors to form catch bonds, which are strengthened rather than weakened by mechanical force. Here we describe a crystal structure of the catch-bond forming bacterial adhesive protein FimH in native fimbrial tips that is dramatically different that previous structures of FimH. The new structure shows how a neighboring domain allosterically inhibits the adhesive domain. In previous crystal structures, the adhesive domain was pre-activated by prevention of these native inter-domain contacts. Molecular dynamic simulations and structural analysis show how mechanical force breaks the native contacts between the autoinhibitory domain and the lectin domain, and how conformational changes in the interdomain region regulate the ligand-binding pocket. These structural changes explain how biochemical and mechanical stimuli affect binding in experiments. Together, these data provides the structural details for how FimH forms allosteric catch bonds. Surprisingly, the FimH adhesive domain has a beta-sandwich motif, a class considered to be structurally rigid. However, different parts of the sandwich can lever open like a pair of pliers, causing large correlated changes in both distal loop regions in spite of only small changes in the fulcrum at the center of the sheets.

Platform AV: Membrane Physical Chemistry II

2817-Plat
Polar Residues in Transmembrane Helices can Dramatically Reduce Mobility on SDS Gels WITHOUT Dimerization
William C. Wimley[1], Kalina Hristova[2], Mikhail Merzliakov[2].
[1]Tulane University Health Sciences Center, New Orleans, LA, USA, [2]Johns Hopkins University, Baltimore, MD, USA.
Polar residues in membrane-spanning helices are known to drive oligomerization in biological and synthetic membranes, as well as in membrane mimetic systems such as detergents. Here we report a study of a hydrophobic peptide that contains either an asparagine (N) or a leucine (L) residue in the 12th position of an otherwise hydrophobic segment of 20 amino acids. These peptides are fully alpha-helical in detergents. In SDS polyacrylamide gels, the L12 peptides migrated as monomers while the N12 peptides always migrated as single bands at twice their apparent molecular weights. In sharp contrast, Forster resonance energy transfer (FRET) experiments in SDS showed little evidence of dimerization of N12 under any conditions studied. Experiments were done with labeled peptides at concentrations up to 50 micromolar and at acceptor to donor ratios from 1:1 to 10:1. SDS concentrations ranged from 3.5 to 70 mM. We also performed in situ FRET experiments on the peptide bands in polyacrylamide gels, where there was little excess FRET observed for the slower N12 bands relative to L12 bands. We conclude that N12 is always monomeric in SDS gels, despite the fact that it appears to migrate as a dimer. Dynamic light scattering experiments showed a significant difference between L12/SDS micelles and N12/SDS micelles. We hypothesize that the polar residue in the center of the otherwise hydrophobic helix alters the interactions between the peptides and detergent and that physical differences in the peptide-detergent micelles, such as shape and stoichiometry are responsible for the altered migration of the N12 peptides relative to the L12 peptides.

2818-Plat
Examining The Role Of Lipid Variations And Proteins On Membrane Biophysics: Synthetic Versus Natural Membrane Vesicles
Florly S. Ariola, **Ahmed A. Heikal**.
Pennsylvania State University, University Park, PA, USA.
Biomembranes in living cells are complex, heterogeneous and dynamic systems that regulate numerous biological processes such as cell signaling,

endocytosis and exocytosis, and protein trafficking. Cholesterol-rich lipid domains have been hypothesized to exist in a liquid-ordered phase and play an important role in cellular functions. Here, we test the hypothesis that cholesterol diffuses as a complex with other lipids in a bilayer. In addition, we examine the role of lipid variation and proteins on the biophysical properties of biomembranes using comparative studies of giant unilamellar vesicles (GUVs) and plasma membrane vesicles (GPMVs) isolated from Hs578Bst live cells. The fluorescence dynamics assay used here includes two-photon fluorescence lifetime imaging, fluorescence resonance energy transfer, and diffusion (both rotational and translational). Different fluorescent lipid analogs are used in these studies to probe both the hydrophobic core and the head group region. Our comparative studies on GUVs and GPMVs serve as a platform to test our understanding of lipid-lipid and lipid-protein interactions in these biomembrane models.

2819-Plat

Two-dimensional Calorimetry: Imaging Thermodynamics and Kinetics of Phase Transitions of Biological Membranes

Antonin Marek, **Alex I. Smirnov**.
North Carolina State University, Raleigh, NC, USA.

Differential scanning calorimetry (DSC) is a relatively rapid and informative biophysical method for studying thermotropic phase behavior of biological membranes. More recently a pressure perturbation calorimetry has been introduced. The latter method is capable of characterizing membrane thermal volume expansion coefficient and kinetics associated with the phase transition. Notably, pressure perturbation calorimetry requires both pressure jump accessory and, most importantly, fast and sophisticated temperature control system to ensure constant sample temperature upon the pressure jump. Here we describe a calorimetry procedure and associated method of data analysis to characterize sample thermal properties as a two-dimensional (2D) object (temperature-time thermal image) whereas data obtained by conventional calorimetry are essentially one-dimensional. In brief, the method utilizes mathematical formalism of the Radon transform (back-projecting algorithm) to separate temperature and time dimensions from a series of thermal flux measurements obtained by a conventional DSC calorimeter at different scanning rates. By this manner static and time-dependent (i.e., relaxation) thermodynamic parameters of an object become resolved and displayed as a single 2D temperature-time thermal image. There are two main advantages of our 2D-calorimetry method: 1) 2D temperature-time thermal image separates and characterizes equilibrium and non-equilibrium thermal properties of a sample; 2) the method improves signal-to-noise ratio for conventional DSC measurements of equilibrium heat capacity as a function of temperature. We demonstrate these advantages of the 2D calorimetry method on examples of imaging thermodynamics and heat relaxation properties of lipid bilayers composed from single and mixed phospholipids with and without cholesterol. We also show that confining lipid bilayers inside nanopores of ca. 175 nm in diameter results in heterogeneous heat transfer kinetics while conventional equilibrium calorimetry curves remain unperturbed. Supported by the DOE Contract DE-FG02-02ER15354.

2820-Plat

Raft recruitment of Membrane Proteins by Native Ligands and GPI-Anchored Proteins: A Model Membrane Study

Ann Kimble-Hill[1], Sumit Garg[1], Amanda Siegel[1], Rainer Jordan[2], **Christoph Naumann[1]**.
[1]Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]Technical University Munich, Munich, Germany.

The role of cholesterol/sphingolipid-enriched lipid heterogeneities, also known as lipid rafts, in membrane protein function represents one of the fascinating topics in cell biophysics and cell biology. Progress in this research area has been slow, due to the small size and transient character of such heterogeneities in plasma membranes of resting cells. To overcome these experimental complications, here we describe two-types of protein-induced membrane protein recruitment processes to/from raft-like domains using a model membrane platform based on a polymer-tethered phospholipid bilayer. The domain-specific quantification of reconstituted membrane proteins in the bilayer is achieved using a combined setup of epifluorescence microscopy and confocal fluorescence correlation spectroscopy. In the first case, we present experimental data concerning the raft recruitment of membrane proteins due to the binding to their native ligands [receptor/ligand pairs investigated: FcγRIII/IgA; urokinase plasminogen activator receptor (uPAR)/ urokinase plasminogen activator (uPA); $\alpha_V\beta_3$ integrin/vitronectin; and $\alpha_5\beta_1$ integrin/fibronectin]. Interestingly, the ligand binding process causes substantial translocations among GPI-anchored proteins (FcγRIII and uPAR) and membrane-spanning receptors ($\alpha_V\beta_3$ and $\alpha_5\beta_1$) to/from raft-like domains. Remarkably, under the experimental conditions chosen, no ligand-induced receptor oligomerization can be observed. In the second case, we explore the impact of uPAR-integrin complex formation on the affinity of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ for lipid rafts. The uPAR/integrin complexation is determined for different ligand states using single molecule fluorescence microscopy. Importantly, our results confirm that such complexes enhance the affinity of integrins for raft domains, thus suggesting a mechanism of GPI protein-induced recruitment of transmembrane proteins to raft domains. Finally, the impact of monolayer and bilayer-spanning raft-mimicking domains on protein recruitment processes is discussed.

2821-Plat

Electromechanical Forces and Flexoelectricity in Plasma Membranes

Ben Harland.
Johns Hopkins, Baltimore, MD, USA.

Flexoelectricity is a phenomenon analagous to piezoelectricity which describes the evolution of electric fields in two dimensional liquid crystals in response to curvature changes (and, conversely, changes in curvature due to applied electric fields). It provides a mechanism for cellular electromechanical transduction in flexoelectric membranes, a process which is important to the physiology of outer hair cells of the inner ear and touch sensitive nerve cells.

We have developed a simple electrostatic model for a bilayer containing anionic phospholipids. Charged ions surrounding the bilayer are distributed according to the Poisson-Boltzmann equation. The correct way to treat the adsorption of positive ions into the bilayer (the ''Stern layer'' of Gouy-Chapman-Stern theory) is not clear. Different regimes are investigated, including a Langmuir Isotherm and a fixed chemical potential approach. It is found that the presence/absense of a flexoelectric effect is critically dependent upon how this adsorption is handled.

Additionally, recent experimental work conducted on cell membrane tethers which are extended in an optical trap setup provides direct estimates for the tensile forces in these tethers as a function of applied voltages. We describe a model which gives quantitative agreement with these measurements.

2822-Plat

Characterizing Changes In The Structure And Orientation Of Supported Model Membranes Upon Binding Of Cholera Toxin B

John Jay Leitch[1], Christa L. Brosseau[1], Sharon G. Roscoe[2], Jacek Lipkowski[1].
[1]University of Guelph, Guelph, ON, Canada, [2]Acadia University, Wolfville, NS, Canada.

Cholera is caused by a protein toxin secreted by the bacterium *Vibrio cholerae*. This toxin is a member of the AB_5 superfamily of toxins composed of an active (A) and a binding (B) pentameric unit. The five binding subunits of the pentamer bind specifically to the GM_1, which is present in the outer leaflet of the host's plasma membrane; however, the mechanism for the translocation of the toxin is not well understood. Model membranes consisting of DMPC+cholesterol+GM_1 were supported on gold electrode surfaces. The changes in the structure and orientation of the model membrane upon binding of the cholera toxin B unit were explored using differential capacitance, chronocoulometry and polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). Changes in the structure and orientation of the toxin protein with respect to a changing electric field were similarly investigated. The IR data suggests that the binding of the toxin to the membrane causes a decrease in the number of *gauche* conformers, which is caused by the constriction of the acyl chains due to interactions between the toxin and the GM_1 glycolipids. The bound toxin induces some minor defects in the membrane; however, these defects are not significant enough to cause measurable changes in the average orientation of the membrane lipids. The major change in the bilayer upon binding of the cholera B unit was a remarkable decrease in the relative hydration of the membrane. This decrease in hydration is mostly like due to the separation of the bilayer from the aqueous electrolyte by the presence of the bound protein layer on the membrane surface. This work is part of ongoing study to understand the mechanism for translocation of the cholera toxin across the plasma membrane.

2823-Plat

COPI Coat Assembly Occurs on Liquid Disordered Domains and the Associated Membrane Deformations are Limited by Membrane Tension

Jean-Baptiste Manneville[1], Jean-François Casella[2], Ernesto Ambroggio[1], Pierre Gounon[3], Julien Bertherat[4], Patricia Bassereau[1], Jean Cartaud[5], Bruno Antonny[2], Bruno Goud[1].
[1]CNRS-Institut Curie, Paris, France, [2]Université de Nice Sophia-Antipolis - CNRS, Valbonne, France, [3]Université de Nice Sophia-Antipolis, Nice, France, [4]Ecole Normale Supérieure, Paris, France, [5]CNRS-Institut Jacques Monod, Paris, France.

Cytoplasmic coat proteins are required for cargo selection and budding of tubulovesicular transport intermediates that shuttle between intracellular